

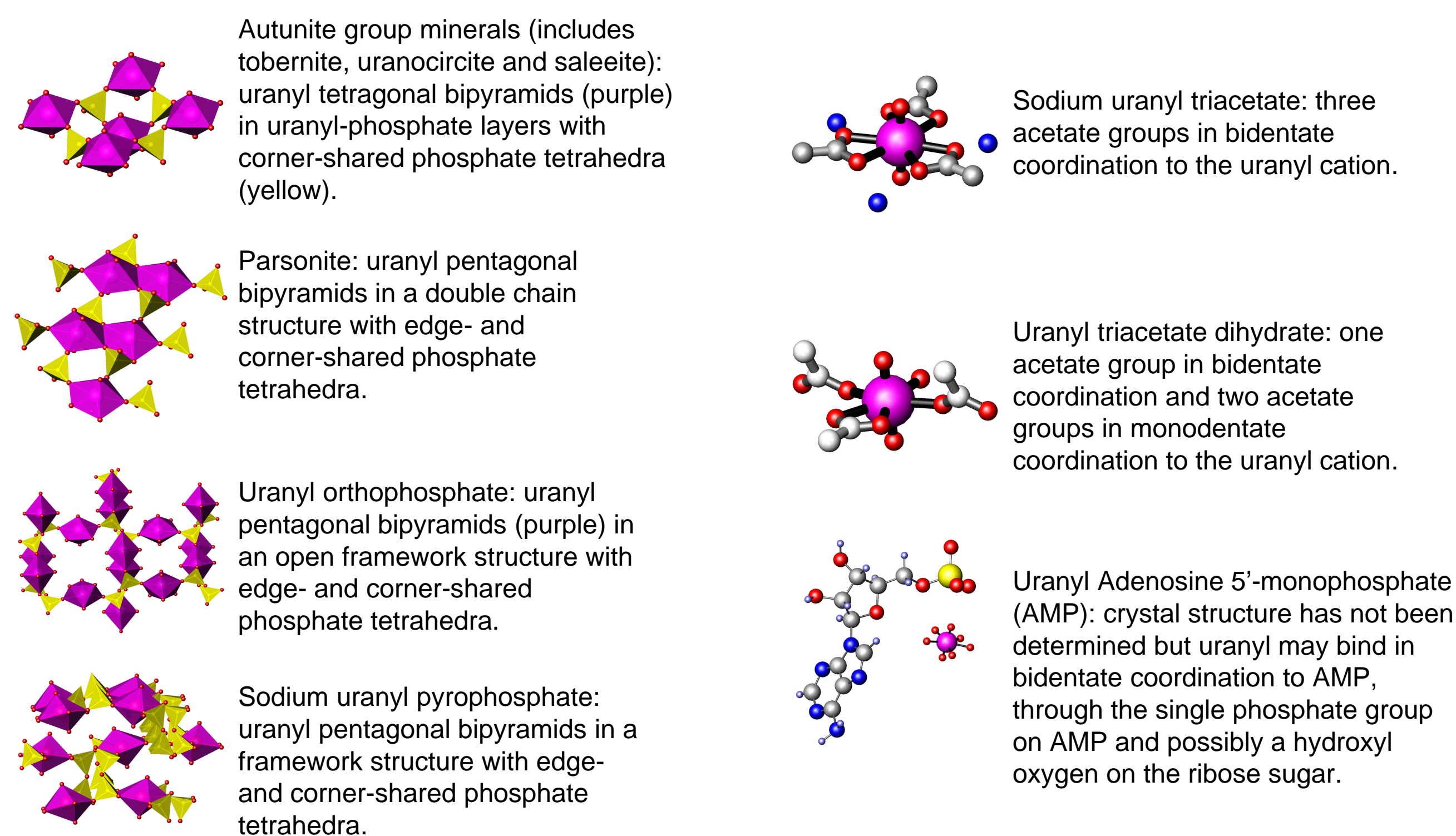
Temperature-Dependant EXAFS Study of Uranium Adsorption to Bacteria

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Introduction

Bacteria play an important role in the fate and transport of radionuclides in the environment. Complexation reactions between surface functional groups on the bacteria and metals affect the distributions of metal contaminants in soils and groundwaters. The exact mechanism of this binding is largely unknown and may change as a function of the specific metal contaminant, the properties of the bacteria and solution conditions. For the case of uranium, titration experiments implicate phosphate and/or carboxylate functional groups as the most likely groups responsible for binding [1,2]. Previous experiments using Extended X-ray Absorption Fine-Structure (EXAFS) spectroscopy have shown that phosphate may be the primary functional group responsible for binding uranyl. In this study, we use extensive temperature-dependant EXAFS spectra of phosphate and carboxylate model compounds and bacteria-sorbed uranium spectra to further investigate this question.

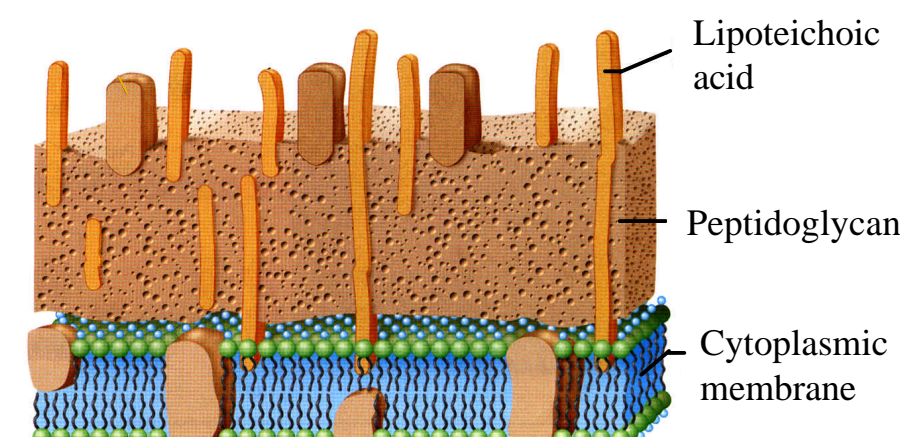
Model Compounds Used



Why use low temperature EXAFS?

EXAFS can provide direct spectroscopic evidence of uranium sorption to functional groups on the bacterial cell surface giving the identity, coordination number and bond distance to neighboring atoms. In practice, poor S/N and back-scattering efficiency limit the usefulness of the data. Low temperature studies provide better EXAFS amplitude S/N and potentially more systematic fitting of the data (from fitting at multiple temperatures). This is especially true in low Z (eg. organic) matrices.

Fig. 1. Schematic showing the overall structure of the gram-positive cell wall. Lipoteichoic acids are acidic polysaccharides attached to the cell wall (and bound to membrane lipids) containing glycerophosphate or ribitol phosphate residues.



from Madigan, Martinko and Parker (2000), pp. 71. Biology of Microorganisms, 9th ed. Prentice Hall.

Materials and Methods

EXAFS spectra were collected on wiggler beamline 4-1 at the Stanford Synchrotron Radiation Laboratory (SSRL) at four temperatures (20, 100, 200 and 300 K) at the uranium L3 edge.

Photoelectron scattering amplitudes and phase shifts were calculated using FEFF 7 code with FEFF input files determined using Atoms (Ravel, 2001) and literature crystal structures. The fitting model generally included only single scattering shells but contributions from the most important multiple scattering shells were included in some fits.

Preparation of Bacterial Samples

Samples of U-238 sorbed to cultures of the gram positive *bacillus sphaericus* were prepared by mixing an aqueous U solution (10^{-6} to 10^{-5} M) with a known biomass at pH 5 in 0.1 M NaCl for 2 hours. Two samples were prepared, one at high U loading and another at low loading.

Results

Fitting of EXAFS Spectra

- fits were made in R-space ($2.5 - 4 \text{ \AA}$), which covers the peak positions of mono- and bidentate phosphorous and carbon, the species expected to bind uranium.
- peak positions of uranium/phosphorous and uranium/carbon shells, monodentate and bidentate can be characteristic but due to overlap (e.g. C_{mono} with P_{mono}) and interference from other scattering peaks (e.g. $U \rightarrow O_{\text{ax}} \rightarrow U \rightarrow O_{\text{ax}}$) very careful EXAFS fitting needs to be done.
- Einstein model fits of the Debye-Waller factor (σ^2) with temperature were examined for their usefulness in improving the fits of the EXAFS spectra.

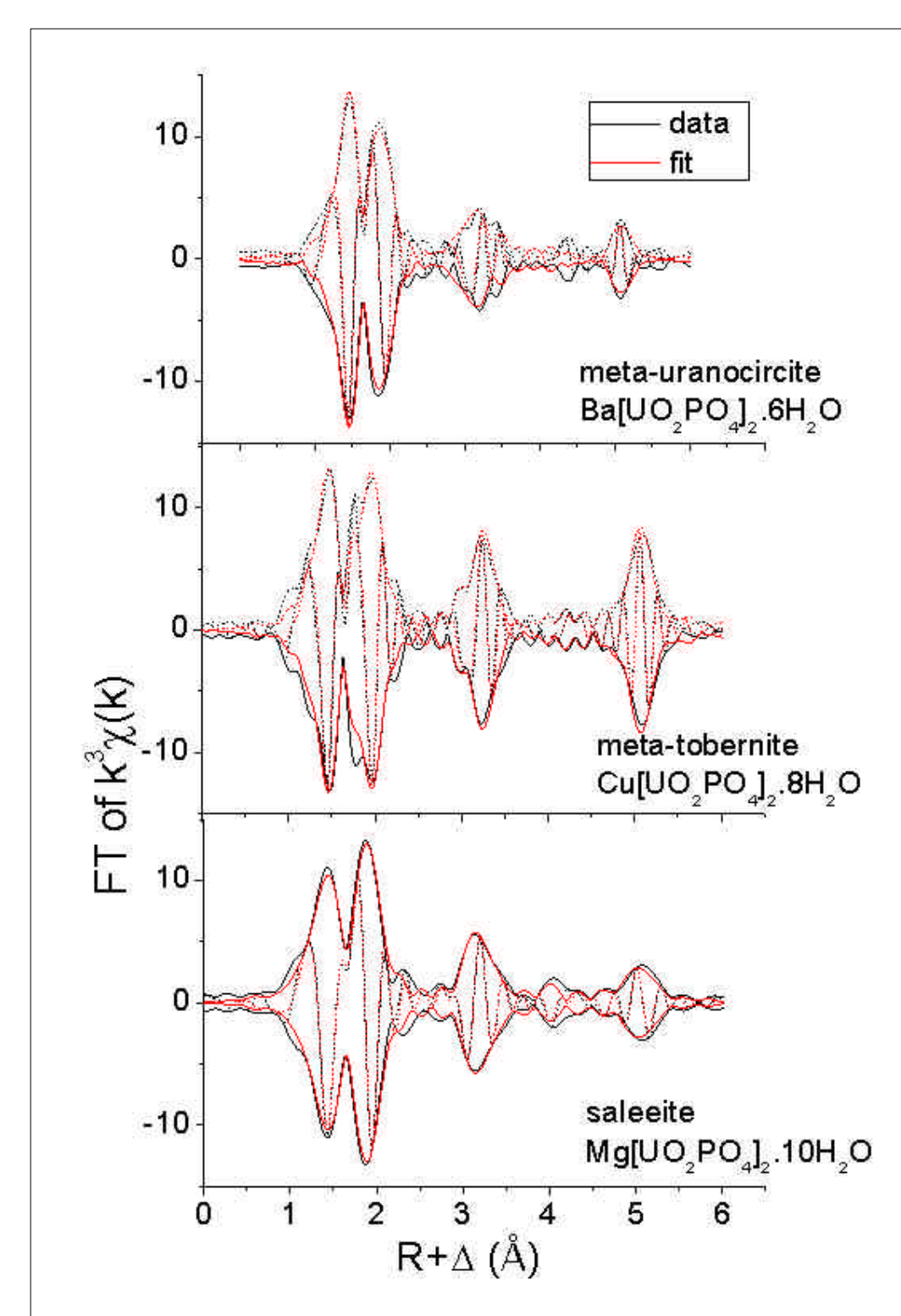


Fig. 2. The magnitude and real part of the Fourier transform of the $k^3\chi(k)$ for some members of the autunite mineral group (left) and other phosphate and carboxylate compounds (right) at $T=20 \text{ K}$. The highlighted regions show the relevant second-shell neighbor (P or C).

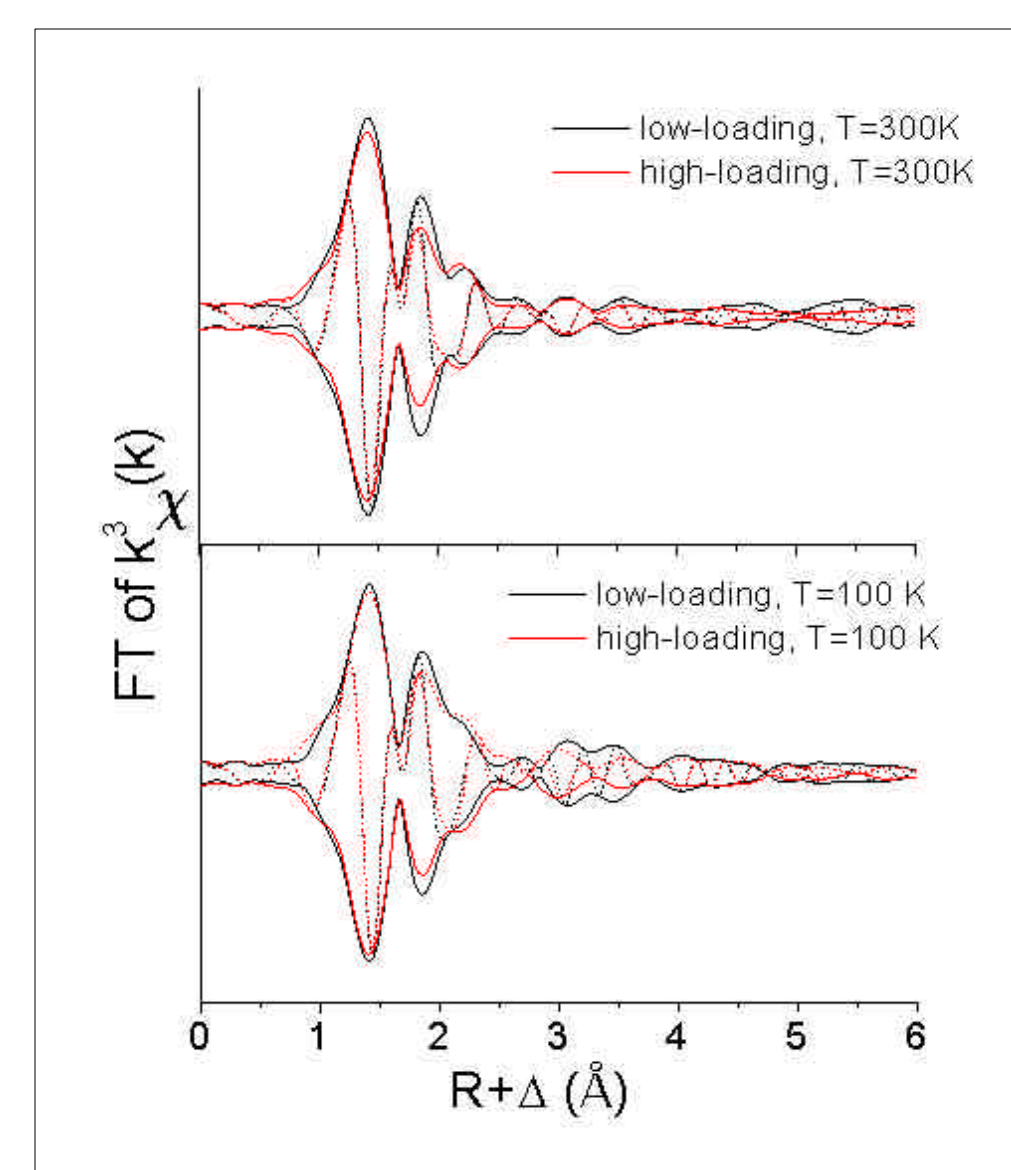
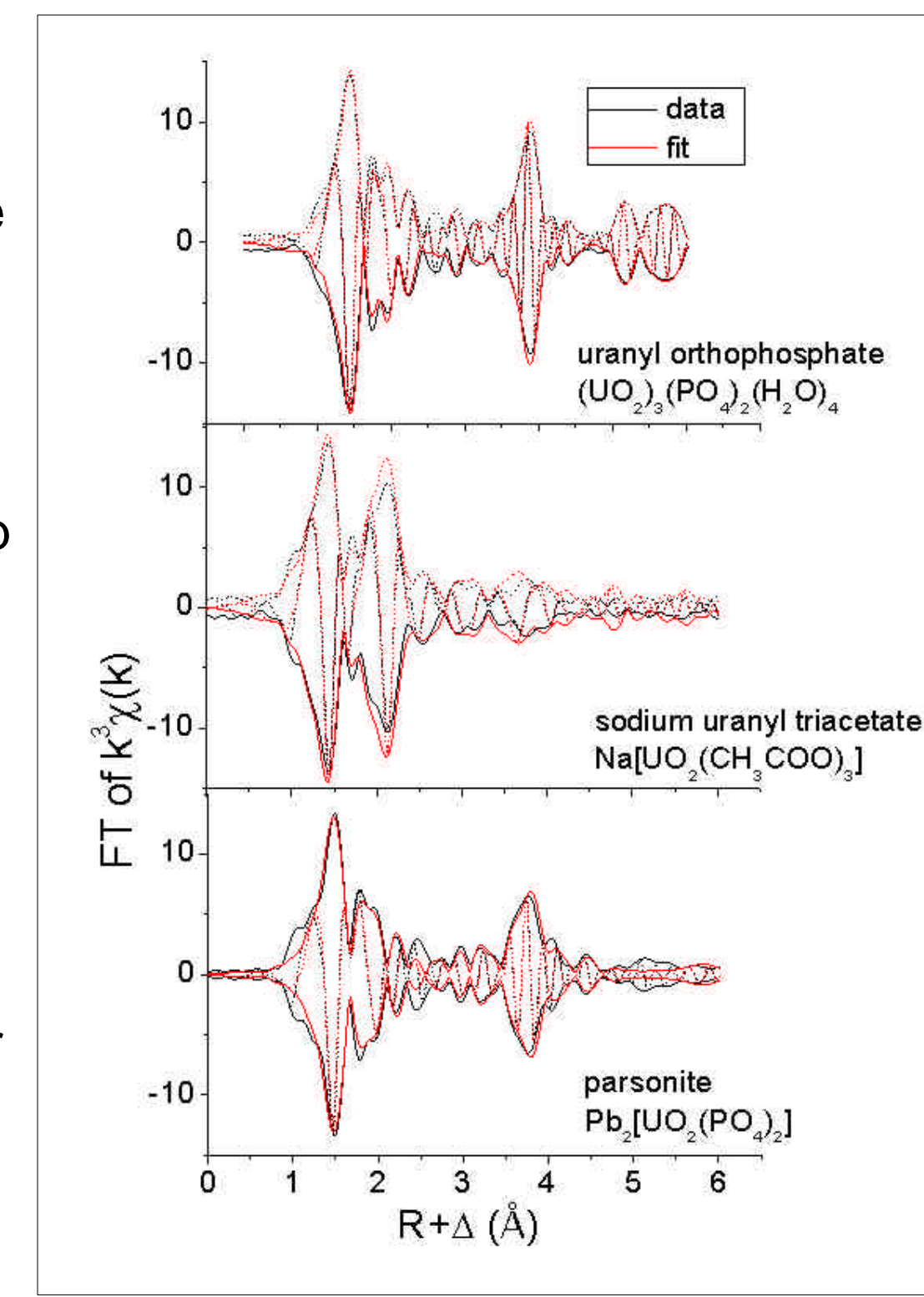


Fig. 3. The magnitude and real part of the Fourier transform of $k^3\chi(k)$ for uranium adsorbed onto *bacillus sphaericus* at pH 5 (left). Best fits to the low loading sample (right).

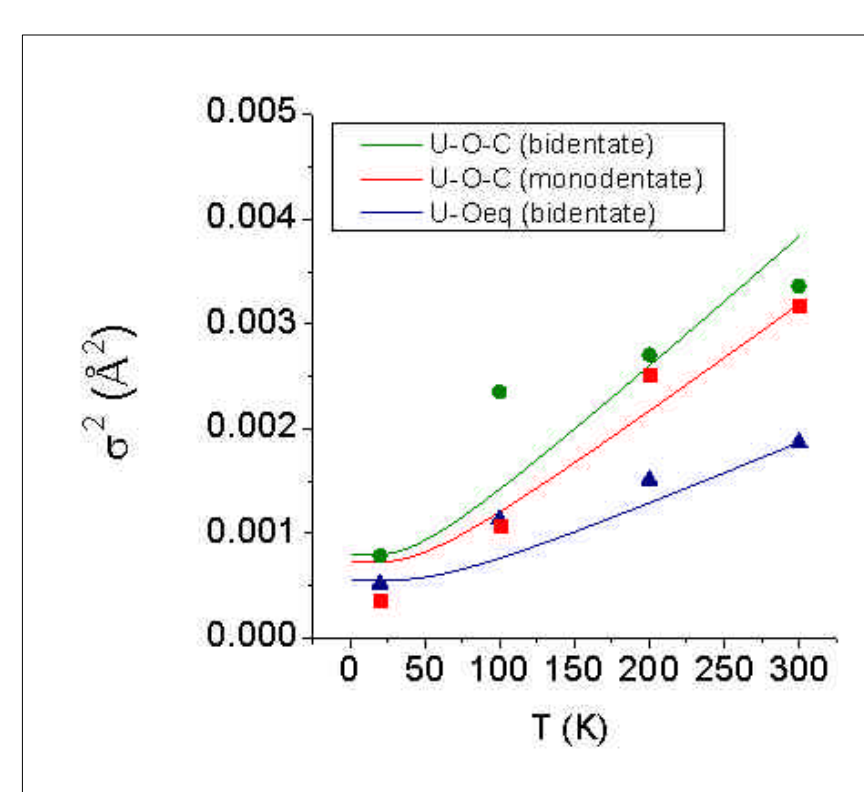
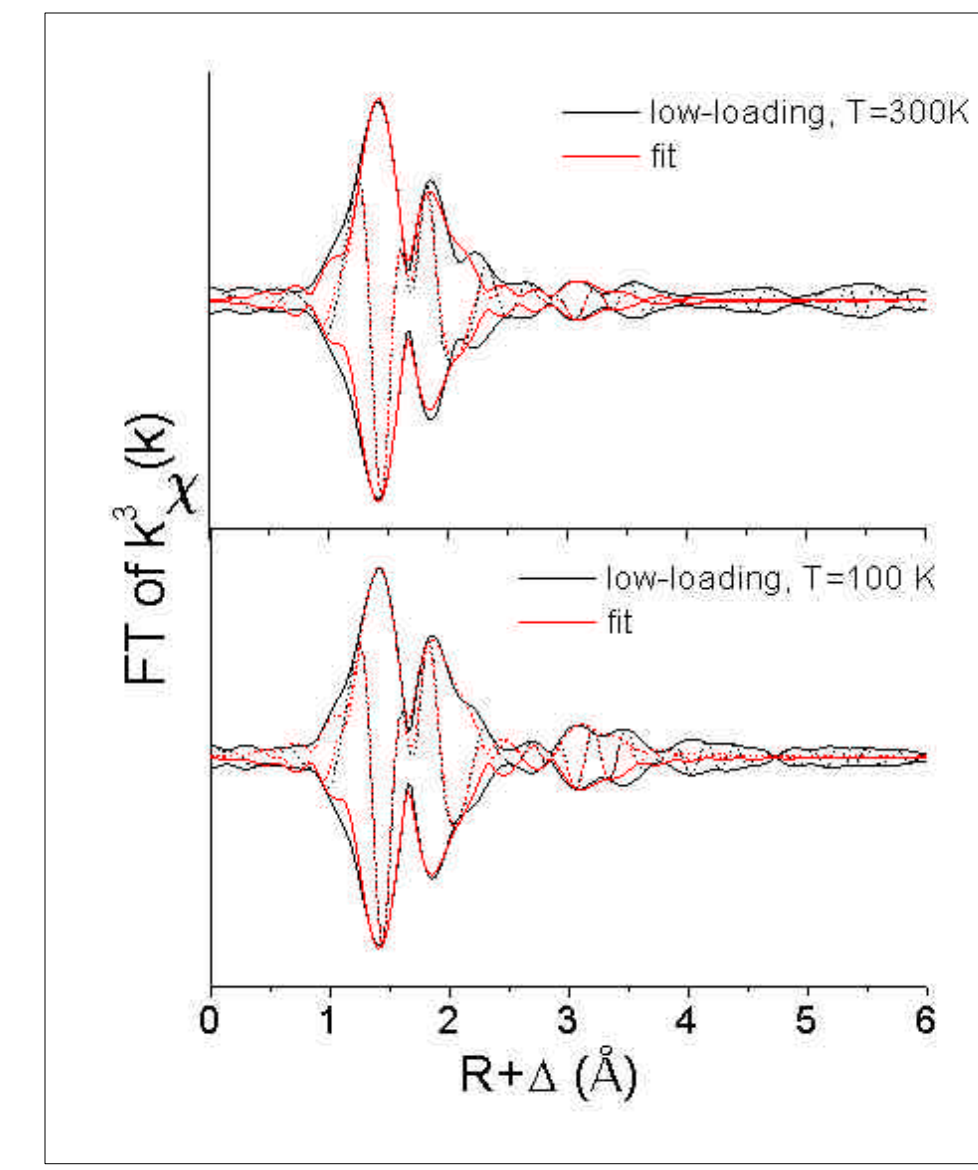
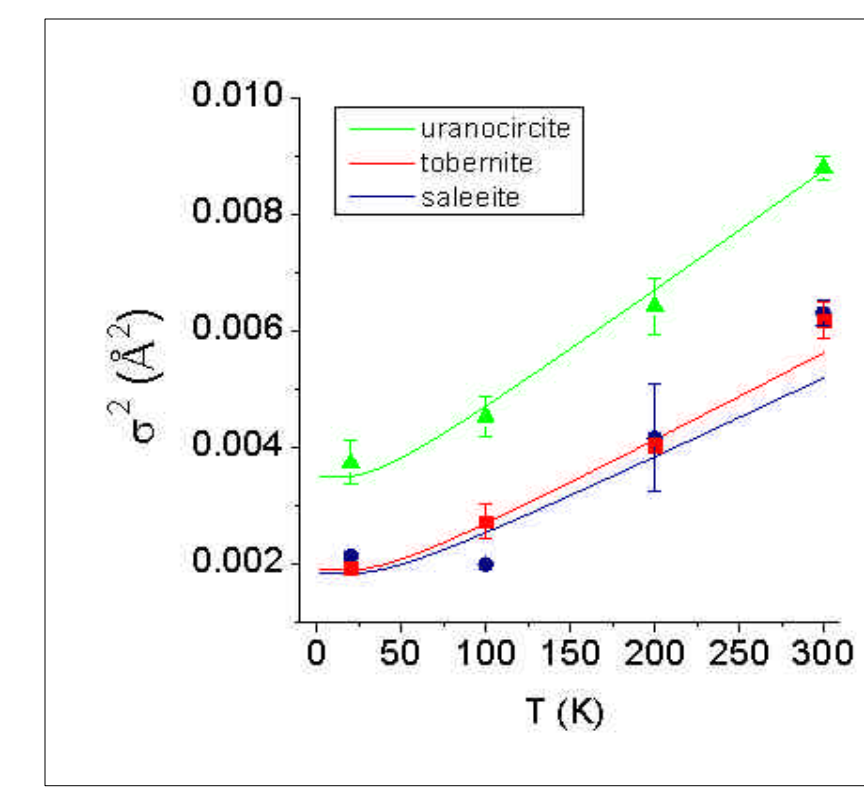


Fig. 4. Einstein model plots for uranyl acetate dihydrate (left) and for the U-P bond in various autunite group minerals (right). A weak temperature dependence indicates a stiffer bond.



Summary

- The adsorption of uranium (UO_2^{2+}) onto bacteria was analyzed using temperature-dependant EXAFS measurements.
- Bacterial adsorption was conducted under both high-loading and low-loading conditions at pH 5 - there was no evidence of precipitation or reduction of uranyl under these conditions.
- Experiments at much lower pH have suggested that phosphate binds uranyl at the bacterial cell surface but at environmentally relevant pH values (5-9) it is possible that complexation involves carbon functionalities.
- Best fit results suggest that phosphate was the dominant binding functional group at pH 5 for the low-loading bacterial samples. The fits for the high-loading samples also suggest phosphate binding but the results are less conclusive and there may significant binding through carbon.
- The best fits overall to the bacterial-adsorbed uranyl were with a single monodentate phosphate binding and/or 1 or 2 monodentate carboxylate (or possibly hydroxyl) groups binding to the uranyl group.
- Fits were made to the uranyl-adsorbed bacteria using 3 single-scattering peaks ($2 \times O_{\text{ax}}$, $6 \times O_{\text{eq}}$ and $1 \times P$) and 1 multiple-scattering peak (P).
- Einstein model fits of the model compounds do not show a significant difference between carbon and phosphorous, in terms of σ^2_{stat} or Einstein temperature, as a second shell neighbor. Therefore, these parameters were not helpful in refining EXAFS fit assignments.

Table 1. Best fit values for the EXAFS spectra of uranyl adsorbed to the bacterial cell using a phosphate model.

Sample	low-loading			high-loading		
	$\Delta R(\text{\AA})$	CN	σ^2	$\Delta R(\text{\AA})$	CN	σ^2
Oax	1.78	2.1	0.0020	1.79	2.2	0.0024
Oeq	2.39	6.3	0.0102	2.41	6.5	0.0130
P	3.55	1.1	0.0017	3.82	1.1	0.0074

Literature Cited

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- Fein, J.B.; Daughney, C.J.; Yee, N.; Davis, T.A. (1997) *Geochim. Cosmochim. Acta*, **61**(16), 3319-3328.

The Future

- We are in the process of looking at organic phosphorous model compounds which will further help in the analysis.
- We plan to do similar measurements at lower pH to see if we agree with existing data and models that indicate that phosphorous dominates uranium binding at low pH.

Acknowledgments

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For further information

Please contact jawarner@lbl.gov. More information on this and related projects can be obtained at <http://lise.lbl.gov/jawarner/ssrl02.pdf>